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Original Article

GENETIC DIVERSITY BETWEEN STEM ROT RESISTANT AND SUSCEPTIBLE GROUNDNUT GENOTYPES USING PCR-RAPD

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ABSTRACT

A study was undertaken to explore the genetic diversity present among different groups of groundnut. Genetic diversity was studied among six groundnut genotypes which varied in their levels of resistance to stem rot caused by Sclerotium rolfsiiSacc. PCR based Random Amplified Polymorphic DNA primers were utilized for the ascertaining the genetic diversity. Genotypes, GG-20 and GG-16 were genotypically distant from remaining four genotypes. Groundnut cultivars, TG-37A, ICGV-86590 were closely related to resistant genotypes, NRCGCS-19 and NRCGCS-319. An overall polymorphism of 91.2% was observed with forty primers where, twenty primers produced 100% polymorphism. The polymorphic bands showed as size ranging from 200 to 1000 bp and the Jaccard's similarity matrix and dendogramindicated NRCGCS-19 and NRCGCS-319 were genetically close with 93% similarity. Hence, PCR-RAPDs may be utilized to cut down the time required for phenotyping of groundnut genotypes against S. rolfsii.

KEYWORDS: Arachishypogaea, Genetic Diversity, RAPD, Sclerotium Rolfsii

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INTRODUCTION

Groundnut(*Arachishypogaea* L.), an important oilseed crop grown in India (Madhusudhana, 2013) with yield of 1367 kg ha^{-1} less than world average (1688 kg ha^{-1}) this may be due to biotic and abiotic stresses, which limit the groundnut production (DGR, 2013).Stem rot incited by *S. rolfsii* causes yield losses up to 27% irrespective of growing seasons (Chohan and Singh, 1974; Tiwari*et al.*, 2004; Ganesan*et al.*, 2007). Suitable cultivar with field resistance to *S. rolfsii*is not available forgroundnut growers in India (Narendrakumar*et al.*, 2013). Until recently, few germplasm are reported with disease tolerance such as, NRCGCS-19, NRCGCS-319 and ICG-12083 (Singh *et al.,* 1997; Bera*et al.,* 2014; Thirumalaisamy*et al.,*2014). Use of chemical and morphological markers failed to explain diversity between closely related species because of environmental effect on it (Matusand Hayes, 2002).

PCR based Randomly Amplified Polymorphic DNAmarkers are good genetic markers because they give rapid results, economicallyconvenient and use small oligonucleotide primers. With a small quantity of template, a very large number of fragmentsare generated from different regions of the genome and hence, multiple loci may be examined very quickly (Kumari*et al.,* 2009).Therefore present study was carried out to characterize the genetic diversity among the stem rot resistant and susceptible genotype of groundnut using PCR based RAPD marker in support of breeder's crop improvement program.

MATERIALS AND METHODS

Six groundnut genotypes *viz.*, NRCGCS-19, NRCGCS-319, GG-20, GG-16, TG-37A and ICGV-86590 were selected for the present study. According to Thirumalaisamy*et al.* (2014) inter-specific crosses, NRCGCS-19 and NRCGCS-319 were resistant, GG-20, GG-16, ICGV-86590 were susceptible to stem rot. Two seeds of each accession were sown in plastic pots filled with sterilized soil and placed in a greenhouse.

Genomic DNA was extracted from fresh leaves (50 mg) of one week old plants by cetyltrimethylammonium bromide (CTAB) method (Doyle and Doyle, 1987). Leaf samples were crushed for 5 min in vial containing 2x CTAB (700 µl) and 2-balls in MP crusher. The mixture was incubated for 30 min at 65 °C followed by addition of equal volumes of chloroform and isoamyl alcohol (25:25 v/v) and centrifugation at 1109 G for 15 min using Beckman Coulter™ Allegra™ X-22R Centrifuge to remove chlorophyll and protein debris. Supernatant was collected in which DNA was precipitated by addition of 1 ml pre-chilled ethanol and separation was done at high speed centrifugation for 5 min. Obtained pellet was washed with 70% ethanol for 2 times and centrifuged at 936 G for 4 min and air dried to get DNA pellet. This will be dissolved in 50 µl Tris-EDTA (TE) and stored at -20 °C till further use. Quality and quantity of DNA was checked in spectrophotometric analysis using a Nano Drop spectrophotometer (ND-1000, Thermo scientific, USA) and also by agarose gel electrophoresis (AGE) using 0.8 per cent agarose.

DNA was amplified using 40 decamer primers which were synthesized at IDT Sterling Biologics (Table 1). The PCR reactions for RAPD were carried out in 25 µl of reaction mixture and cycle condition as described by Mondal *et al.* (2007). Each reaction mixture contained 10 ng μl^{-1} DNA template, 2 µl dNTPs (2 mM), 2.5µl Taq buffer(10x) with 15 mM $MgCl_2$, 1.25 µl Primer (20 p moles/µl), 0.5 µl Taq polymerase enzyme (3 unit μl^{-1}), and PCR water 16.75 µl. PCR reaction conditions adopted are; initial denaturation at 94 °C for 5 min, 35 cycles at 92 °C for 1 min, primer annealing at 37 °C for 30 sec, primer extension at 72 °C for 2 min and final extension at 72 °C for 5 min, followed by cooling to 4 °C until recovery of the samples. The amplified products were analyzed on agarose gel (1.2%) containing 0.2 µg ml^{-1} of ethidium bromide in 1x Tris-Acetate-EDTA (TAE) buffer (pH 8.0) and visualized using Fujifilm FLA-5100 scanner. For the comparison, known DNA marker of 100 bp ladder was used.

Each amplification product was considered an RAPD marker. Gels were scored on the basis of the presence (1) or absence (0) of each band for all isolates. All amplifications were repeated at least twice and only reproducible band were considered for analysis.The data matrix thus generated was used to calculate Jaccard's similarity coefficient for each pair-wise comparison. The similarity coefficient was calculated in silico using formula given by Jaccard (1908) *i.e.*,

$$Similarity\ coefficient = A/N$$

Where, A=number of matching bands for each pair of comparisons. N= number of bands in two sample observed.

The similarity coefficients were subjected to Unweighted Pair-Group Method of Arithmetic Averages (UPGMA) cluster analysis to group the isolates based on their overall similarities. NTSYSpc version 2.02i Software (Rohlf, 1997) was used for cluster analysis and subsequent dendrogram preparation.

RESULTS AND DISCUSSIONS

To ascertain the genetic diversity among six groundnut genotypes screened for resistance against *S. rolfsii*. PCR-RAPD used as molecular marker and all the 40 primers showed polymorphism ranging from 70 to 100 per cent (Table 1).

A total of 362 polymorphic bands were obtained out of 397 bands which indicated 91.2% polymorphism with all 40 primers and our findings are in line with observations of Vyas*et al.* (2014) where 51.8% overall polymorphism was obtained with thirteen RAPD primers. The banding pattern of amplified DNA samples ranged from 200-1000 bp in size (Figure 1)with an average of 9.925 bands per primer, higher than the findings of Vyas*et al.* (2014) who reported 4.2 average bands per primer.Difference in band intensity occurs due to each primer hybridizes in different extents to target DNA and the undefined target DNA may exist in multiple copies per genome. Similar results have already been reported by Skroch and Nienhuis (1995) that RAPD bands amplified by one primer vary in intensity from those amplified by another primer. Twenty primers *viz.*, OPP3, OPP4, OPP5, OPP6, OPP8, OPP9, OPP11, OPP12, OPP13, OPP16, OPP19, OPP20, OPB2, OPB3, OPB4, OPB8, OPB13,OPB16, OPB18 andOPB19were noted with maximum polymorphism (100.0%). Remaining twenty primers were witnessed with polymorphism ranging from 70.0 to 90.9 per cent where primer OPB-5 was reported with minimum polymorphism.

Dendogram derived from UPGMA resulted in two main clusters in which GG-20 separated from other groundnut genotypes with 27% similarity only (Figure 2).The remaining genotypes, GG-16, TG-37A, ICGV-86590, NRCGCS-19 and NRCGCS-319 were clustered into another group. In second cluster, GG-16 showedcomparatively more diversity with TG-37A, ICGV-86590, NRCGCS-19 and NRCGCS-319 and there was 79% dissimilarity observed between GG-20 and GG-16. Remaining genotypes showed closely related genetic makeup however only 81% similarity was observed in TG-37A and ICGV-86590 whereas NRCGCS-19 and NRCGCS-319 was very much parallel with 92% similarity. The latter two genotypes were developed by crossing cultivars with wild relatives, *i.e.*¸ they are inter-specific hybrids (Bera*et al*., 2014). Vary low or no polymorphism in cultivated types to abundant polymorphism in wild *Arachis* has been reported by Halward*et al.* (1991). However, Siva *et al.* (2014) and Al-Saghir and Abdel-Salam (2015) detected low genetic diversity of peanut cultivars using RAPD markers.

The variation between tested genotypes was mainly due to their genetic background which depends on their pedigree as well as botanical types as reported by Herselman (2003). Groundnut cultivars, GG-20 and GG-16 belongs to Virginia Bunch and Virginia Runner habitat, respectively while, ICGV-86590 and TG-37A were Spanish Bunch types (Rathnakumar*et al.*, 2013).Jaccard's similarity co-efficient matrix revealed that, very negligible similarity of less than 0.3 was observed between GG-20 and rest of genotypes (Table 2). However, GG-16 also showed 0.36, 0.38, 0.39 and 0.40 similarities respectively with genotypes, TG-37A, ICGV-86590, NRCGCS-19 and NRCGCS-319. Genotype, TG-37A shared nearly 80% similarity with ICGV-86590, NRCGCS-19 and NRCGCS-319where ICGV-86590 had nearly 90% similarity with the latter two genotypes. Jaccard's similarity co-efficient of 0.93 was registered between NRCGCS-19 and NRCGCS-319 suggesting their genetic closeness.

CONCLUSIONS

Genetic diversity among the groundnut cultivars was well explained by PCR based RAPD primers with higher polymorphism level. Both resistant genotypes, NRCGCS-19 and NRCGCS-319 were genetically close while, GG-20, GG-16, TG-37A and ICGV-86590 were genetically dissimilar. For quick, repetitive and economical results, RAPDs may be utilized in crop improvement programmes.

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APPENDIXES

Table 1: Arbitrary Primer Sequence and Level of Polymorphism in Groundnut Genotypes

Sr. No.	Primer	Sequence (5'-3')	Total No of Band (a)	Polymorphic Band (b)	Polymorphism (%) (b/a×100)
1	OPP1	GTAGCACTCC	10	8	80.0
2	OPP2	TGGGCACGCA	9	7	77.8
3	OPP3	CTGATACGCC	10	10	100.0
4	OPP4	GTGTCTCAGG	10	10	100.0
5	OPP5	CCCCGGTAAC	10	10	100.0
6	OPP6	GTGGGCTGAC	10	10	100.0
7	OPP7	GTCCATGCCA	10	8	80.0
8	OPP8	ACATCGCCCA	10	10	100.0
9	OPP9	GTGGTCCGCA	10	10	100.0
10	OPP10	TCCCCGCTAC	9	8	88.9
11	OPP11	AACGCGTCGG	7	7	100.0
12	OPP12	AAGGGCGAGT	7	7	100.0
13	OPP13	GGACTGCCTC	9	9	100.0
14	OPP14	CCAGCCGAAC	11	9	81.8
15	OPP15	GGAAGCCAAC	8	7	87.5
16	OPP16	CCAAGCTGCC	9	9	100.0
17	OPP17	TGACCCGCCT	8	7	87.5
18	OPP18	GGCTTGGCCT	14	12	85.7
19	OPP19	GGGAAGGACA	9	9	100.0
20	OPP20	GACCCTAGTC	9	9	100.0
21	OPB1	GTTTCGCTCC	10	9	90.0
22	OPB2	TGATCCCTGG	10	10	100.0
23	OPB3	CATCCCCCTG	10	10	100.0
24	OPB4	GGACTGGAGT	10	10	100.0
25	OPB5	TGCGCCCTTC	10	7	70.0
26	OPB6	GTCTCTGCCC	9	7	77.8
27	OPB7	GGTGACGCAG	10	8	80.0
28	OPB8	GTCCACACGG	12	12	100.0
29	OPB9	TGGGGGACTC	11	10	90.9
30	OPB10	CTGCTGGGAC	10	9	90.0
31	OPB11	GTAGACCCGT	8	6	75.0
32	OPB12	CCTTGACGCA	12	9	75.0
33	OPB13	TTCCCCCGCT	12	12	100.0
34	OPB14	TCCGCTCTGG	11	10	90.9
35	OPB15	GGAGGGTGTT	12	9	75.0
36	OPB16	TTTGCCCGGA	12	12	100.0
37	OPB17	AGGGAACGAG	12	10	83.3

Table 1: Contd.,

38	OPB18	CCACAGCAGT	9	9	100.0
39	OPB19	ACCCCCGAAG	11	11	100.0
40	OPB20	GGACCCTTAC	7	6	85.7
TOTAL			**397**	**362**	**91.2**

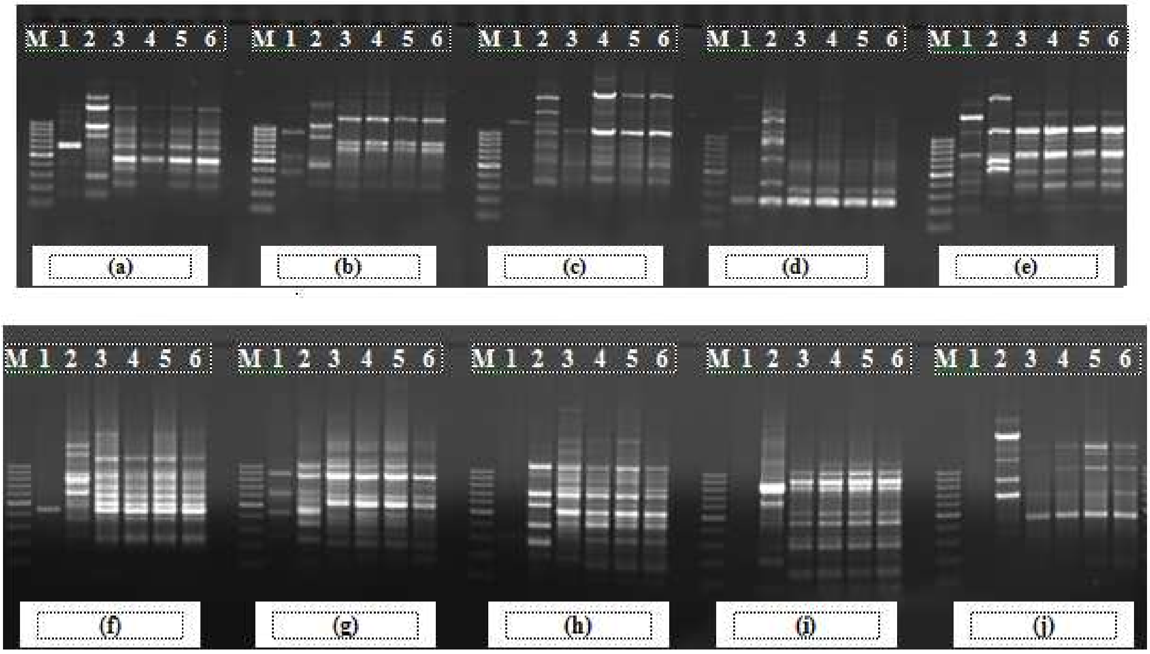

Figure 1: Polymorphic Bands Obtained by PCR-RPD Profiling of Genotypes (a), OPB-1, (b) OPB-2, (c) OPB-3, (d) OPB-4, (e) OPB-5, (f) OPP-6, (g) OPP-7, (h) OPP-8, (i) OPP-9 and (j) OPP-10 and where M Denotes Marker (100 Bp Ladder), 1=GG-20, 2=GG-16, 3=TG-37A, 4=ICGV-86590, 5=NRCGCS-19 and 6=NRCGCS-319

Table 2: Jaccard's Similarity Coefficient Matrices for Six Groundnut Genotypes Based on RAPD Profiling

	GG-20	GG-16	TG-37A	ICGV-86590	NRCGCS-19	NRCGCS-319
GG-20	1.00					
GG-16	0.21	1.00				
TG-37A	0.29	0.36	1.00			
ICGV-86590	0.27	0.38	0.82	1.00		
NRCGCS-19	0.28	0.39	0.82	0.88	1.00	
NRCGCS-319	0.27	0.40	0.84	0.90	0.93	1.00

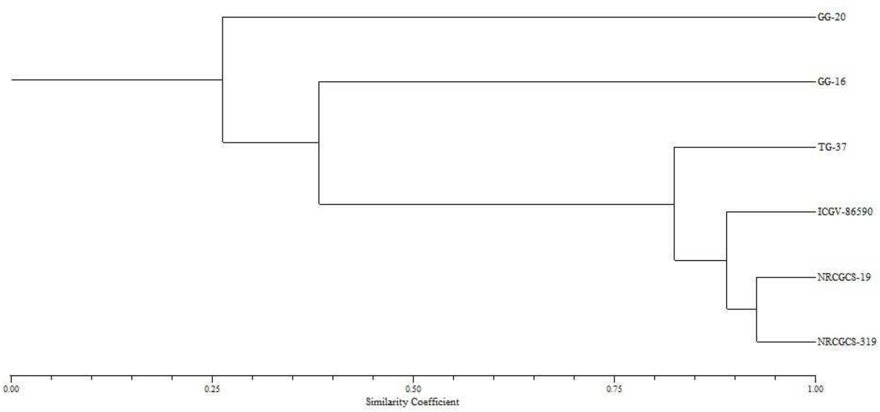

Figure 2: Dendogram of Tested Genotypes Derived from UPGMA Procedure using Jaccard's Coefficient